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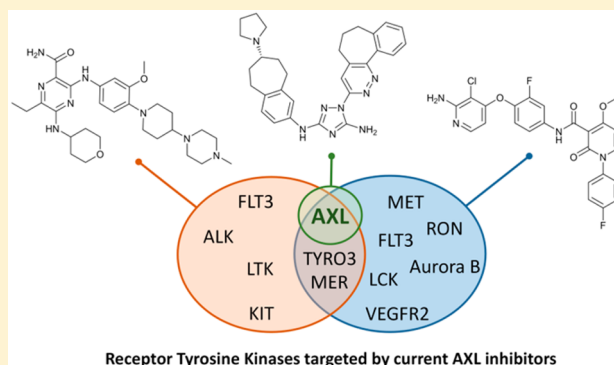
AXL Inhibitors in Cancer: A Medicinal Chemistry Perspective

Miniperspective

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ABSTRACT: Dysregulation of the AXL receptor tyrosine kinase has been associated with many types of cancer. It has not been until recently, however, that targeting AXL has come under the spotlight because of ever accumulating evidence of its strong correlation with poor prognosis and drug resistance. The entry of the first AXL-branded inhibitor in clinical trials in 2013 marked an important milestone for the clinical validation of AXL as an anticancer target. Nevertheless, to weigh the current contribution and potential future impact of AXL inhibition in the clinic, it is fundamental to recognize that several kinase inhibitors approved or in clinical development have AXL as either a prominent secondary or even the primary target. Through this review, the chemical and biological properties of the main inhibitors targeting AXL (either intentionally or unintentionally) will be discussed, along with the prospects and challenges to translate AXL inhibitors into a bona fide therapeutic option.



Receptor Tyrosine Kinases targeted by current AXL inhibitors

1. INTRODUCTION

Receptor tyrosine kinases (RTKs) are multidomain transmembrane proteins that function as sensors for extracellular ligands. Ligand receptor binding induces receptor dimerization and activation of their intracellular kinase domain, leading to the recruitment, phosphorylation, and activation of multiple downstream signaling cascades.¹ To date, 58 RTKs have been identified in the human genome, which regulate a broad range of cellular processes, including cell survival, growth, differentiation, proliferation, adhesion, and motility.^{1,2}

In 1991 Bryan and co-workers were the first to report the discovery of an unidentified transforming gene, eventually named AXL, from two patients with chronic myeloid leukemia (CML).³ The AXL protein (also known as UFO, ARK, and TYRO7) was later classified as a RTK belonging to the TAM (TYRO3, AXL, and MER) subfamily. Since then, overexpression or overactivation of the AXL protein has been correlated with the promotion of multiple tumorigenic processes. High levels of AXL expression have been associated with poor prognosis in different cancers such as glioblastoma multiforme,⁴ breast⁵ and lung⁶ cancer, osteosarcoma,⁷ and acute myeloid leukemia.⁸ In addition, it has been demonstrated that abnormal activation of AXL signaling is one prominent mechanism by which tumor cells undergo epithelial–mesenchymal transition (EMT)^{9,10} and develop drug resistance to both targeted therapies^{10–12} and chemotherapy.^{13,14} EMT is a process consisting of multiple biochemical changes by which polarized epithelial cells gain a mesenchymal cell phenotype, leading to increased migration and invasive properties. In cancer, EMT biomarkers are a hallmark of invading cells and

consequently cancer metastases.^{9,10} Accumulating evidence supporting the role of AXL in cancer together with the first entry of a selective AXL inhibitor in clinical trials¹⁵ has brought AXL inhibitors to the center of pharma interests. As some excellent articles have recently reviewed the biological role of AXL in cancer,^{16–19} the aim of this review is to complement them by focusing on the most significant medicinal chemistry developments and challenges found on the pharmacological targeting of AXL kinase activity with small molecules.

2. THE AXL/GAS6 AXIS

2.1. Structure of AXL and Its Major Ligand GAS6.

Proteins within the TAM family contain a β -stranded N terminal lobe consisting of two immunoglobulin (Ig) like domains and two fibronectin type III (FTIII) repeats which comprise the extracellular region. This is connected via a transmembrane region to a helical C terminal lobe that contains an intracellular kinase domain¹⁹ (Figure 1a). TAM family members TYRO3 (aka BRT, DTK, RSE, SKY, and TIF) and MER (aka MERTK, RP38, and TYRO12) can bind to two vitamin K-dependent extracellular ligands, protein S and the growth arrest specific protein 6 (GAS6). Of these two ligands, AXL has been shown to bind only GAS6, a secreted protein of 75 kDa (Figure 1a).¹⁹ The affinity of TAM proteins for the ligand GAS6 is different, following the ranking of affinity AXL > TYRO3 > MER. AXL possesses around 10-fold superior affinity for GAS6 than MER.¹⁷ Apart from protein S and GAS6,

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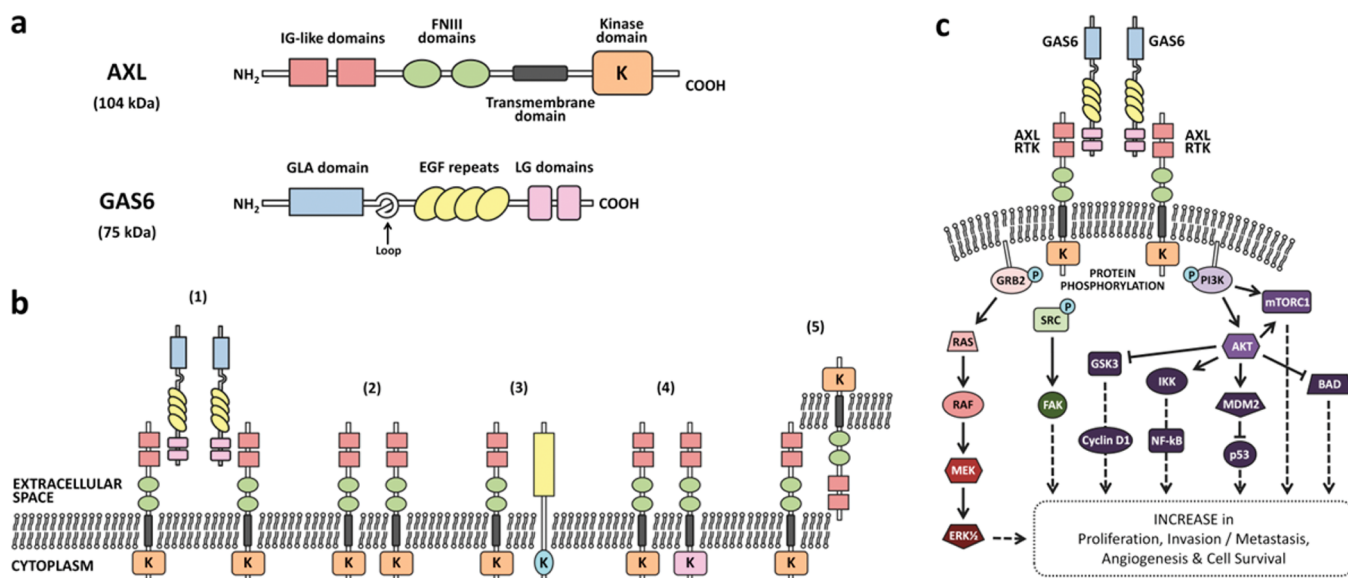


Figure 1. (a) AXL kinase and its extracellular ligand GAS6. (b) Types of AXL activation: (1) GAS6 ligand-dependent activation of AXL; (2) ligand independent activation of AXL; (3) heterophilic activation of AXL with a non-TAM family protein; (4) heterophilic activation of AXL with MER or TYRO3; (5) transcellular ligand independent activation of AXL. (c) Downstream AXL signaling pathways upon classical GAS6-mediated activation.

additional extracellular ligands for TAM receptors have been recently discovered such as TUBBY, TULP-1, and galectin-3,^{20,21} while evidence so far suggest that AXL can only be activated by GAS6 and TULP-1.²⁰

2.2. Receptor Activation and Downstream Signaling.

AXL can be activated by a variety of mechanisms (Figure 1b), the most common being the ligand dependent activation in which AXL binds with GAS6 to form a dimer complex consisting of two AXL molecules bound to two GAS6 molecules. Other activation mechanisms can occur such as ligand independent activation,²² which tends to happen when AXL is greatly overexpressed or under oxidative stress, e.g., ligand independent AXL activation in vascular smooth muscle cells.²³ Heterophilic activation of AXL with either TYRO3 or MER has been hypothesized. While there is no evidence yet of AXL/MER interactions,²⁴ it has been recently shown that AXL and TYRO3 heterodimerize or coexist in the same molecular complex in chronic lymphocytic leukemia B-cells.²⁵ GAS6-independent heterophilic dimerization with non-TAM kinases such as VEGFR1 has also been observed,²⁶ which represents an alternative resistance mechanism developed in response to anti-VEGFR1 therapy. Lastly, transcellular homophilic binding of the extracellular domains of AXL has been proven, where the extracellular binding of the AXL protein causes cell aggregates leading to increased AXL kinase activity.²⁷ Under physiological conditions, maximal stimulation of TAM RTKs including AXL is achieved in the presence of the specific protein ligand and an extracellular lipid moiety, the lipid phosphatidylserine (PtdSer). While PtdSer is an abundant phospholipid ubiquitously present in the membrane of all cells, it is mostly found in the inner layer, pointing its polar head toward the cytoplasm, due to the action of a class of enzymes called flippases.²⁸ PtdSer becomes available to activate TAM receptors only when externalized on the cell membranes in apoptotic cells, aggregating platelets, exosomes and invading virus envelopes.¹⁹

Dimer formation promotes autophosphorylation of tyrosine residues on the intracellular region of AXL, most prominently on Y779, Y821, and Y866.¹⁹ The phosphorylated kinase domain then activates downstream proteins by cross-

phosphorylation, and various signaling cascades take place. The main signaling pathways activated by the GAS6/AXL axis are shown in Figure 1c. AXL activation causes phosphorylation of GRB2, which subsequently activates the RAS-RAF-MEK-ERK pathway leading to cancer cell proliferation.²⁹ AXL also phosphorylates PI3K, thus leading to increased levels of phosphatidylinositol trisphosphate and resulting in recruitment and activation of AKT. AKT is then responsible for the activation of multiple prosurvival proteins, such as IKK, MDM2, or mTOR, and the inactivation of proapoptotic signals such as the BCL-2 family member BAD. Direct AXL-mediated phosphorylation of SRC or through crosstalk with other RTK and the subsequent activation of FAK leads to the promotion of migration and invasiveness.¹⁹ As mentioned, apart from downstream pathways, AXL has also been shown to exhibit crosstalk with numerous RTK such as VEGFR,²⁶ EGFR,³⁰ and MET,³¹ which highlights the complexity of the regulatory role of the AXL receptor and underlines the plausible involvement of AXL in different mechanisms of drug resistance observed in some cancers. In addition, AXL and other TAM proteins play a significant role in inflammatory processes;¹⁹ this is due to the phosphorylation of AXL causing the upregulation of SOCS proteins and STAT-1.³² The importance and implications of the TAM family in inflammatory events and disease will be further discussed in the last section of this review.

2.3. AXL in Cancer. Overexpressed levels of AXL have been found in numerous cancer types and in most cases high AXL expression correlates with a poorer patient prognosis. AXL gene expression is mediated by SP1/SP3 transcription factors and modulated by CpG methylation of its promoter.³³ In addition, the expression of the AXL receptor is post-transcriptionally regulated by two specific microRNAs, miR-34a and miR-199a/b, the expression of which is suppressed by promoter methylation in several solid tumors (e.g., non-small-cell lung cancer (NSCLC), colorectal cancer, breast cancer).³⁴

In NSCLC, interest in AXL as a potential oncology target has risen in recent years due to the involvement of AXL upregulation in resistance to EGFR-targeted therapy¹⁰ along with evidence of AXL crosstalk with EGFR.³⁰ EGFR-driven

Table 1. Summary of the Main AXL Inhibitors and Their Target Kinases

compd	drug (code name)	primary target	AXL inhibition	developer/sponsor	phase of development ⁵⁶
1	Foretinib (XL880, GSK1363089)	MET, VEGFR2, AXL, RON	IC ₅₀ (in vitro) = 11 nM ⁵¹ IC ₅₀ (in cells) < 100 nM ⁴⁴	GSK	Phase II, active, not recruiting
2	Cabozantinib (XL184, BMS-907351, marketed as Cometriq)	VEGFR2, MET, MEK, KIT, RET, AXL	IC ₅₀ (in vitro) = 7 nM ⁵⁷ IC ₅₀ (in cells) = 42 nM ⁵⁷	Exelixis Inc./various, including NCI and Memorial Sloan Kettering Cancer Center	Approved for medullary thyroid cancer Phase II and III for different solid tumors
3	Merestinib (LY2801653)	MET, MST1R, DDR1, TIE1, MER, TYRO3, AXL	IC ₅₀ (in vitro) = 11 nM ⁵⁹ IC ₅₀ (in cells) = 2 nM ⁵⁹	Eli Lilly and Co.	Phase I, active, recruiting
4	MGCD265	MET, AXL, VEGFR2	Data for AXL not reported	Mirati Therapeutics	Phase I, active, recruiting
5	ASLAN002 (BMS-777607)	AXL, RON, MET, TYRO3, MER, FLT3	IC ₅₀ (in vitro) = 1.1 nM ⁵²	Bristol-Myers Squibb/Aslan Pharmaceuticals	Phase I, active, not recruiting
6	NPS-1034	AXL, DDR1, FLT3, KIT, MEK, MET, ROS1, and TIE1	IC ₅₀ (in vitro) = 10 nM ⁶¹ IC ₅₀ (in cells) < 0.5 μ M ⁶¹	NeoPharm	Preclinical
7	LDC1267	MET, AXL, TYRO3	IC ₅₀ (in cells) = 19 nM ⁶²	Lead Discovery Centre	Preclinical
8	Bosutinib (SKI-606, marketed as Bosulif)	BCR-ABL, ABL, SRC, YES, MEK, AXL, BMX	IC ₅₀ (in vitro) = 0.56 μ M ⁶⁶ IC ₅₀ (in cells) = 0.34–1.65 μ M ⁶⁶	Pfizer	Approved for CML with resistance to treatment Phase II and III for different CML stages
9	Gilteritinib (ASP2215)	FLT3 and mutants, ALK, AXL	IC ₅₀ (in vitro) < 1 nM ⁶⁸	Astellas Pharma Global	Phase I/II, active, recruiting
10	SGI-7079	MET, MER, YES, RET, FLT3, AXL	IC ₅₀ (in vitro) = 58 nM ⁹ IC ₅₀ (in cells) < 1 μ M ⁷⁰	Tolero Pharmaceuticals/Astex Pharmaceuticals	Preclinical
11	TP-0903	Aurora A and B, JAK2, ALK, ABL, AXL, MER	IC ₅₀ (in vitro) = 27 nM ⁷¹ IC ₅₀ (in cells) = 222 nM ⁷¹	Huntsman Cancer Institute/Tolero Pharmaceuticals	Preclinical
12	Crizotinib (PF-02341066, marketed as Xalkori)	ALK, MET, RON, AXL	IC ₅₀ (in vitro) = 294 nM ⁷²	Pfizer/Further clinical sponsors include NCI, EORTC, etc.	Approved for NSCLC Phase II/III for different solid tumors
13	Amuvatinib (MP470)	KIT, PDGFR1, FLT3, RET, AXL	IC ₅₀ (in cells) < 1 μ M ⁷⁹	Astex Pharmaceuticals	Phase II, development discontinued
14	UNC2025	MER, FLT3, AXL, TYRO3	IC ₅₀ (in vitro) = 1.6 nM ⁸²	University of North Carolina	Preclinical
15	S49076	MET and mutants, AXL, MER, FGFRs	IC ₅₀ (in vitro) = 7 nM ⁸⁵ IC ₅₀ (in cells) = 56 nM ⁸⁵	Institut de Recherches Internationales Servier	Phase I, active, recruiting
16	Sunitinib (SU11248, marketed as Sutent)	KIT, FLT3, PDGFR, VEGFR2, AXL	IC ₅₀ (in vitro) = 9 nM ⁸⁷	Pfizer/Further clinical sponsors include NCI, Baylor Breast Care Center, M.D. Anderson Cancer Center, etc.	Approved for renal cell carcinoma, imatinib-resistant gastrointestinal stromal tumor, and metastatic pancreatic neuroendocrine tumors Phase II/III for different solid tumors
17	BGB324 (R428)	AXL (selective)	IC ₅₀ (in vitro) = 14 nM ⁵³ IC ₅₀ (in cells) < 30 nM ⁵³	Rigel Pharmaceuticals/BerGen BIO	Phase I/II, active, recruiting

tumors are typically treated with erlotinib (structure not disclosed), a potent EGFR inhibitor; however, these tumors can develop resistance with one major cause of this thought to be associated with AXL overexpression.³⁵ It has been demonstrated that continuous EGFR inhibition ultimately induces GAS6 upregulation, hence causing increased AXL activity. These data are further supported by increased levels of AXL, GAS6, and protein S being found in over 50% of NSCLC cell lines, with AXL and MER being the most phosphorylated RTKs in NSCLC cells.³⁶ AXL upregulation tends to correlate with lymph node metastasis and poorer patient survival.³⁷ In vitro knockdown studies have shown that inhibition of AXL causes a decrease in the growth of NSCLC cells and a significant reduction of migration and invasiveness.³⁸

AXL is generally expressed at low concentrations in healthy breast tissue, while in the advanced stages of breast cancer levels of AXL expression are significantly increased, particularly in ER+ cells,³⁹ and correlate with a highly invasive phenotype.⁴⁰ In breast cancers, overactivation of AXL signaling is further enhanced by the progesterone receptor B through upregulation of GAS6 and it has been reported that HER2 can also activate AXL signaling.⁴¹ Lorens and co-workers showed that RNAi knockdown of AXL in triple negative breast cancer models reduces tumor growth and metastases in mice.⁴² Other studies have also shown the importance of AXL inhibition in breast cancer cell migration.⁴³ The pathological role of AXL in breast cancer is also evidenced by its association with resistance to lapatinib (structure not disclosed) in HER2+ breast cancer.⁴⁴

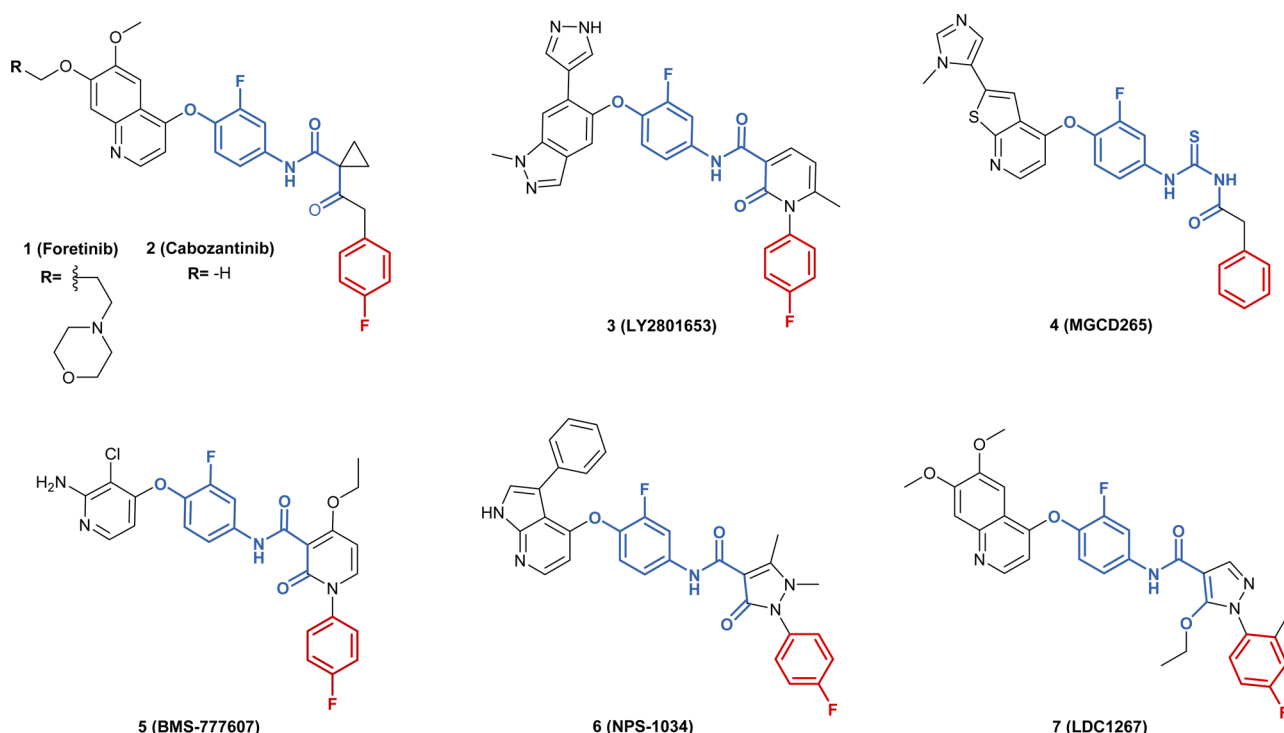


Figure 2. Structures of type II kinase inhibitors in clinical and preclinical development that target the AXL RTK.^{49,51,52,59–62} The hinge-binding heterocycles are displayed at the left side of the structures. The chemically related structural motifs that connect the “head” heterocycle with the terminal phenyl ring (in red) are highlighted in blue. Note that all compounds but 4 present a fluoro atom at the para position of the terminal phenyl ring.

Several studies have found high levels of phosphorylated AXL and GAS6 in many glioblastoma cell lines, which are biomarkers that generally correlate with poorer prognosis and cancer recurrence.³ In vivo studies have shown that tumor growth is significantly reduced in tumors that have AXL inhibited, showing a less invasive phenotype with an increased overall survival of the animals.⁴⁵ Keating et al. also found that AXL mRNA levels and protein expression are elevated in astrocytic patient samples and cell lines.⁴⁶ RNAi-mediated silencing of AXL RTK expression led to a significant increase in chemosensitivity of astrocytic cells (including glioblastoma cells) in response to temozolomide, carboplatin, and vincristine treatment (structures not disclosed), suggesting scope for combination therapy with a variety of traditional cytotoxic agents.⁴⁶

In prostate cancer there has been shown to be a direct correlation between the malignancy of the disease and GAS6/AXL expression in undifferentiated metastatic human prostatic cancer cells, which leads to activation of AKT and MAPK phosphorylation.⁴⁷

Originally discovered in AML (acute myeloid leukemia), AXL has therefore been long proposed as a therapeutic target for this disease. AML cells induce secretion of GAS6 by bone marrow–derived stromal cells which then activates AXL, stimulating cell survival and drug resistance.⁴⁸ It has been shown that inhibition of AXL in AML results in the improvement of clinically relevant indicators, suggesting AXL inhibitors could be used on their own or in combination therapy to treat AML.

3. AXL INHIBITORS

While a wide range of kinase inhibitors that target the AXL RTK have been described in the literature (see list in Table 1),

in most cases AXL was not the intended primary target but a secondary consequence of the similarities among the kinase domains of AXL and other RTK such as MET or MER. Consequently, many of those inhibitors often exhibit less potency for AXL than against its main target. Inhibitors falling into this category are cabozantinib (2)⁴⁹ and bosutinib (8),⁵⁰ drugs approved by the FDA for the treatment of medullary thyroid cancer and CML, respectively, or the drug candidate foretinib (1).⁵¹ Interestingly, the opposite case has also been described, e.g., the MET-branded inhibitor BMS-777607 (5),⁵² the potency of which is 3-fold greater against AXL than against MET in vitro. The first inhibitor reported to be purposely developed as an AXL selective inhibitor was BGB324 (17),⁵³ which also holds the questionable honor of being considered the first AXL inhibitor to enter clinical trials.¹⁵

It is important to note that much of the kinase inhibition data available in the literature were generated in vitro using the corresponding recombinant proteins. While cell-free assays provide a practical screening tool for in-house drug discovery programs, reported biochemical data need to be used with caution due to the lack of correlation with cell activity. Consequently, inhibitory activity in cells (rather than biochemical data) represents the optimal data source to directly compare the potency of different inhibitors. Unfortunately, the activity of kinase inhibitors in cells is not always reported. Biochemical and cell activity data (when available) of the main AXL inhibitors reported to date are compiled in Table 1.

Since the discovery of allosteric AXL inhibitors has not yet been reported, all kinase inhibitors described in this review are ATP-competitive inhibitors having either type I or type II binding modes. Type I inhibitors bind to the ATP site of the kinase in its active conformation (aspartate–phenylalanine–glycine (DFG) motif oriented toward the active site or DFG–

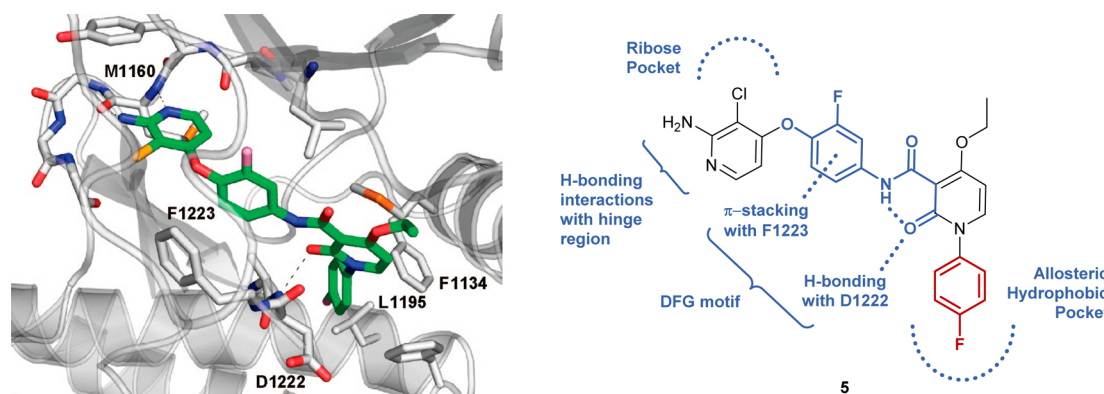


Figure 3. Cocystal structure of **5** bound to the inactive conformation of MET kinase domain (left panel)⁵² and main binding interactions of the inhibitor (right panel).

in), being the most common mode of binding. Both type I and type II inhibitors typically contain an adenine-mimicking heterocyclic moiety that binds to the hinge region of the ATP site. However, type II inhibitors adopt an extended conformation via interactions with DFG residues of the activation loop to enter an allosteric region (adjacent to the ATP binding site) that is only accessible in the inactive DFG-out conformation of the kinase.⁵⁴ Therefore, while the complex formed by type II inhibitors and the inactive conformation of the kinase impede substrate-enzyme binding (=enzymatic competition), ATP molecules and type II inhibitors bind to different conformations of the kinase catalytic site. The type II binding mode is generally attributed to provide inhibitors that display superior selectivity profile. However, such a general assumption has been disputed by some authors.⁵⁴

3.1. Type II Kinase Inhibitors. Compound **1** is a MET/VEGFR2 inhibitor (IC_{50} = 0.4 and 0.9 nM in cell-free assays, respectively) in clinical development (Figure 2). Cocrystallization of **1** with the MET kinase domain shows that the inhibitor occupies both the ATP binding site and an adjacent pocket of the inactive conformation of the kinase.⁵⁵ After validation of its safety in healthy volunteers during phase I,⁵¹ **1** went on to stage II clinical trials for lung, papillary renal carcinoma, hepatocellular carcinoma, and refractory gastric cancer. All trials but one have now been completed.⁵⁶ No phase III trials have yet been initiated. While the anticancer properties of compound **1** are attributed to MET and VEGFR2 inhibition, **1** inhibits other kinases such as AXL at the low nanomolar level (IC_{50} = 11 nM in cell free assay).⁵¹ Inhibitor **1** has been reported to lead to almost complete inhibition of AXL phosphorylation in lapatinib-resistance breast cancer cells at 100 nM and to restore sensitivity to lapatinib (structure not disclosed) in treated cells.⁴⁴

2 is an extremely potent VEGFR2 inhibitor (IC_{50} = 0.035 nM, in cell-free assays) that also inhibits a wide number of kinases, including MET, KIT, RET, and AXL, in the low nanomolar range.⁵⁷ In 2012 the FDA gave approval for its use in medullary thyroid cancer with orphan drug status.⁴⁹ A number of clinical trials sponsored by different parties are ongoing for a wide variety of indications, including NSCLC and cancers of prostate, kidney, breast, pancreas, and ovaries.⁵⁶ This orally available drug inhibits the kinase activity of human recombinant MET and AXL with IC_{50} = 1.3 and 7 nM, respectively, i.e., 35- to 200-fold lower potency than for its main target VEGFR2 in biochemical assays. On the basis of the cell-free inhibition data of **2**, this compound could in principle be

considered a selective VEGFR2 inhibitor. In cells, however, **2** inhibits phosphorylation of VEGFR2, MET, and AXL at much closer concentrations (IC_{50} values of 1.9, 7.8, and 42 nM, respectively),⁵⁷ which delineates the limitations of relying only on biochemical data to evaluate and rank on- and off-target activities. A phase II study (NCT01639508) with inhibitor **2** is currently ongoing in patients with advanced NSCLC whose tumors present one of the following gene changes: RET, ROS1, NTRK fusion or increased MET or AXL activity.⁵⁶ A randomized, open-label phase III study of **2** versus everolimus (structure not disclosed) in patients with advanced renal cell carcinoma has just been reported (NCT01865747).⁵⁸ The study concluded that progression-free survival was longer with inhibitor **2** than everolimus (structure not disclosed) for patients that had relapsed after VEGFR-targeted therapy and highlighted the multitargeting capabilities of the drug as partly responsible for its excellent response rate.

As shown in Figure 2, the chemical structures of inhibitors **1** and **2** are almost identical. Significant structural similarities are also found in related kinase inhibitors such as LY2801653 (**3**),⁵⁹ MGCD265 (**4**),⁶⁰ **5**,⁵² NPS-1034 (**6**),⁶¹ and LDC1267 (**7**)⁶² (see chemical moieties highlighted in blue and red in Figure 2), which may explain why all these compounds strongly inhibit a common subset of RTKs, including MET, VEGFR2, MER, and AXL. Inhibitor **3** is a potent MET inhibitor that targets several RTKs (MST1R, MER, TYRO3, AXL, etc.) at the low nanomolar range both in biochemical assays and in cells (Table 1).⁵⁹ Eli Lilly and Co. has recently completed phase I trials for inhibitor **3** in healthy volunteers and is currently recruiting for a safety study in patients with advanced cancer in combination with cisplatin, cetuximab, gemcitabine, and ramucirumab (structures not disclosed).⁵⁹ The goal of this phase I study is to find the recommended phase II dose of **3** that may be safely given to patients with selected advanced cancers in combination with chemotherapy and/or immunotherapy. Another related drug candidate, **4**, a potent inhibitor of MET, VEGFR-2, and other RTKs (IC_{50} = 1–10 nM, in biochemical assays),⁶⁰ also entered several phase I studies with healthy volunteers and patients with advanced malignancies under the sponsorship of Mirati Therapeutics. A trial is active and recruiting to evaluate the safety of daily doses of the drug in advanced cancer patients. Preliminary results of this study reported that a NSCLC patient with AXL gene amplification responded very well (~50% lesion reduction) after just four daily doses of **4**,⁶³ indicating that certain AXL abnormalities may be clinically relevant oncogenic drivers in NSCLC.

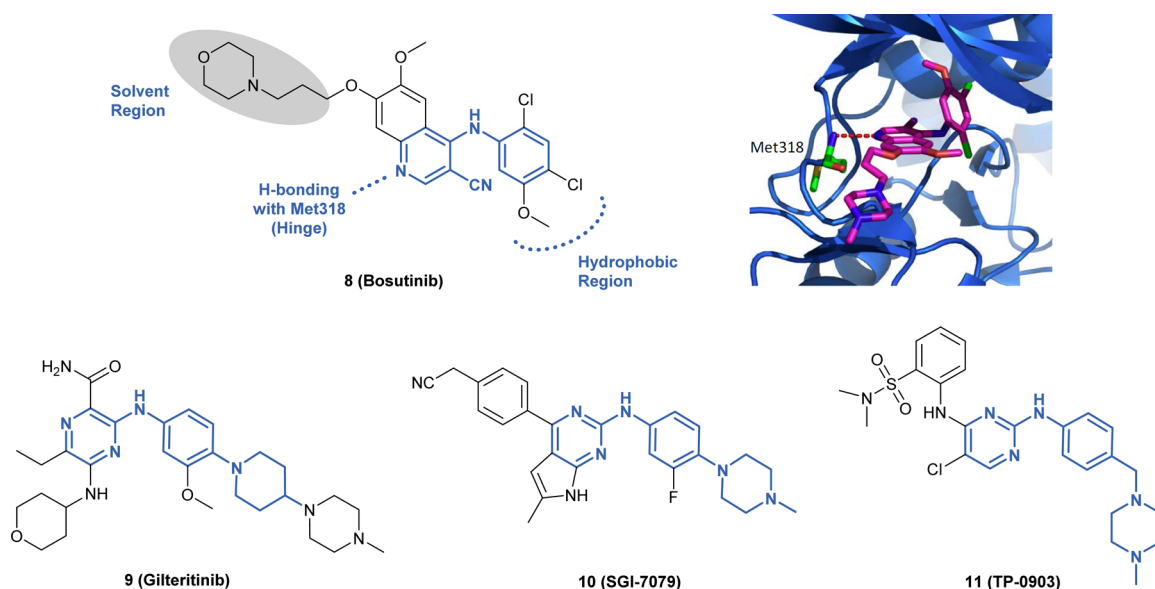


Figure 4. Chemical structures of multikinase type I inhibitors 8–11 and cocrystal structure of 8 bound to the active conformation of ABL kinase (reproduced from *Trends in Pharmacological Sciences*, Vol. 36, Wu, P.; Nielsen, T. E.; Clausen, M. H. FDA-approved small-molecule kinase inhibitors, pp 422–439, Copyright 2015, with permission from Elsevier⁶⁷). Shared chemical motifs are highlighted in blue.

5 is a drug candidate developed by Bristol Myers-Squibb that strongly inhibits MET, AXL, and MER RTKs. X-ray crystal structure of **5** complexed with the MET kinase domain shows that the inhibitor binds at the ATP pocket of the MET kinase with the activation loop in a DFG-out (inactive) conformation (see Figure 3).⁵² The 2-aminopyrimidine core anchors the inhibitor to the hinge region via two hydrogen bonds, whereas the central phenyl ring and 2-pyridone group interact by π -stacking and H-bonding with the phenylalanine and glutamate residues, respectively, of the DGF motif (Figure 3). Analogous mode of binding was observed for inhibitors **1** and **3**. While **5** was rationally designed to inhibit MET (IC_{50} = 3.9 nM, in biochemical assays), its inhibitory potency against AXL is actually higher (IC_{50} = 1.1 nM, in biochemical assays),⁵² meaning that **5** is the most potent in vitro inhibitor that has the AXL kinase as its primary target. This compound went through phase I and II clinical trials for advanced or metastatic solid tumors. While the results have not yet been reported, Aslan Pharmaceuticals is now sponsoring a new phase I study in cancer patients to identify the maximum tolerated dose of **5**.⁵⁶

Besides the above-mentioned drugs and clinical candidates, two additional structurally related inhibitors targeting AXL have recently been reported, **6**⁶¹ and **7**.⁶² Both compounds contain some of the key features found in other previously mentioned AXL inhibitors, i.e., the central phenyl ring and the terminal *p*-fluorophenyl moiety in this case connected through a carboxamidopyrazole linker (Figure 2), groups that are likely to be involved in type II binding.⁶⁴ While these compounds are still in preclinical development, they have displayed remarkable activities against chemoresistant cell lines and metastasis. Compound **6**, developed by NeoPharm, shows significant inhibitory activity against multiple RTK including AXL, DDR1, FLT3, KIT, MEK, MET, ROS1, and TIE1. IC_{50} against human recombinant MET and AXL was 48 and 10 nM, respectively.⁶¹ **6** demonstrated inhibition of AXL signaling in NSCLC cells with acquired resistance to EGFR-tyrosine kinase inhibitors. Significantly, treatment of these resistant cell lines with **6** restored the sensitivity of the cells to gefitinib and erlotinib

(structures not disclosed), resulting in induction of cell death.⁶¹ Inhibitor **7** inhibits the three members of the TAM family in cells at low nanomolar levels. Treatment of wild-type natural killer (NK) cells with **7** efficiently enhanced antimetastatic NK cell activity in vivo.⁶²

3.2. Type I Kinase Inhibitors. Inhibitor **8** is used in the clinic for the treatment of adult patients with CML after resistance or intolerance to prior therapy due to its capacity to inhibit most imatinib-resistant forms of the BCR-ABL fusion protein. However, **8** is a promiscuous kinase inhibitor that also inhibits other proteins such as SRC, ABL, MEK, and BMX with IC_{50} = 1–10 nM.⁶⁵ Through screening of chemical libraries in the search for inhibitors of AXL signaling, Zhang et al. discovered that **8** also inhibits GAS6-mediated tyrosine phosphorylation of AXL in Hs578T breast cancer cells (IC_{50} = 0.34 μ M, similar levels to those found for SRC inhibition in the same cell line) and inhibited cell motility and invasiveness.⁶⁶ In this respect, the inhibitory properties of compound **8** can be considered unique, since it targets both receptor and nonreceptor tyrosine kinases that are dysregulated in many types of cancer. Studies by Yuan et al.⁵⁰ have shown that **8** mediates inhibition of AXL signaling in hepatocellular carcinoma cells and decreases their invasiveness by suppressing the expression of the EMT-inducing transcription factor SLUG, further suggesting that the distinct polypharmacological properties of **8** may become beneficial in the treatment of various cancer indications and, in particular, metastasis.

As shown in Figures 2 and 4, the chemical similarities between the structures of compound **8** and inhibitors **1** and **2** are remarkable. The three inhibitors contain a 6-methoxyquinoline core with substitutions on the positions C4 and C7 of the heterocycle. Inhibitor **1** can be considered a hybrid molecule between **2** and **8**, since it has a morpholinopropoxy group at the C7 position, like **8**, and it shares with **2** the same substituted fluorophenyl group at the C4 position of the quinoline. While all three compounds contain a central phenyl group that, in principle, could π -stack with the Phe of the inactivation loop of the enzyme, compound **8** lacks the key cyclopropyl-1,1-

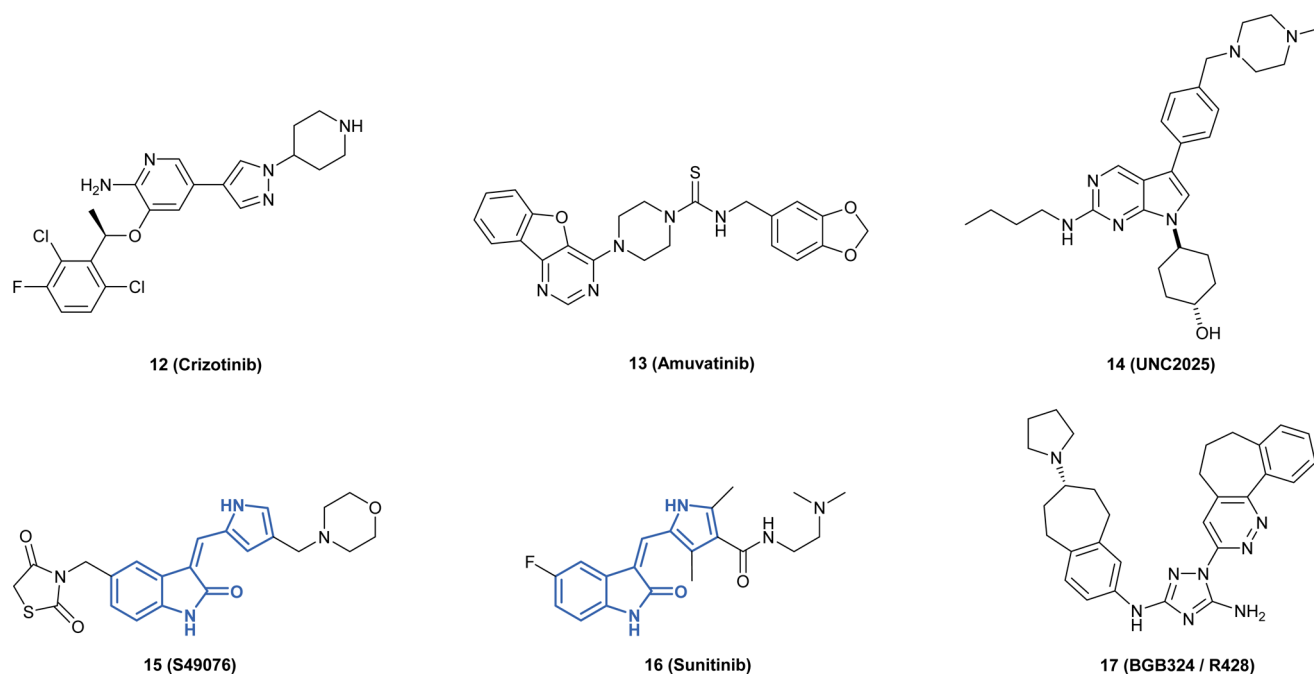


Figure 5. Structures of type I AXL inhibitors 12–17. Shared chemical motifs between compounds 15 and 16 are highlighted in blue.

dicarboxamide linkage and the terminal *p*-fluorophenyl group required to access the hydrophobic pocket of the DFG-out conformation of kinases. According to the cocrystal structure of **8** with the ABL kinase domain (Figure 4),⁶⁷ compound **8** is a type I kinase inhibitor. Interestingly, compound **8** shares some structural similarities with other inhibitors of the AXL RTK, such as gilteritinib (**9**),⁶⁸ SGI-7079 (**10**),⁹ and TP-0903 (**11**)⁷¹ (see Figure 4). Although there is not yet any X-ray structural information or biochemical assay addressing the mode of binding of **9**–**11** to AXL or related kinases, based on their structures they are likely to be type I inhibitors. The differences in AXL activity among these compounds could become useful to initiate novel medicinal chemistry campaigns.

Compound **9** is a highly potent inhibitor of FLT3 (including mutant forms) and AXL with subnanomolar IC₅₀.⁶⁸ The compound exhibits potent antileukemic activity against AML with either or both FLT3-ITD and FLT3-D835 mutations.⁶⁹ Under the sponsorship of Astellas Pharma, **9** is currently undergoing clinical trials (phase I/II) to assess its safety and tolerability, including the maximum tolerated dose alone and in combination with chemotherapy, in subjects with relapsed or treatment-refractory AML.⁵⁶

10 is a relatively potent AXL inhibitor (IC₅₀ = 58 nM, in cell-free assays) in preclinical development that also targets MET, MER, YES, RET, and FLT3 at a similar concentration range.⁹ **10** inhibits GAS6-stimulated AXL signaling in inflammatory breast cancer cells (IC₅₀ < 1 μM), resulting in decreased cell proliferation and invasion.⁷⁰ Byers et al. at the MD Anderson Cancer Center identified AXL as a potential therapeutic target for overcoming EGFR inhibitor resistance associated with the mesenchymal phenotype and demonstrated that treatment with inhibitor **10** sensitized lung cancer cells to erlotinib (structure not disclosed), particularly in an acquired resistance setting.⁹

11 is an AXL-branded inhibitor developed by Bearss and co-workers at the Huntsman Cancer Institute⁷¹ that is currently under preclinical development by Tolero Pharmaceuticals. The compound, which was rationally designed using a homology

model of the AXL active site, inhibits AXL with an IC₅₀ = 27 nM (biochemical assays) but also other kinases such as Aurora A and B, JAK2, ALK, and ABL at even lower concentrations. In fact, cell cycle studies have revealed that the inhibitor induced strong cell cycle arrest by inhibition of Aurora A and B.⁷¹ Recent studies have shown that **11** is effective in inducing apoptosis of B-cell chronic lymphocytic leukemia (CLL) cells as a single agent at nanomolar concentrations and found a synergistic effect with BTK inhibitors.²⁵ In the same work they demonstrated that CLL B-cells not only express constitutively active AXL but also increased levels of TYRO3, although AXL was found to be the predominant TAM RTK regulating CLL B-cell survival.

Crizotinib (**12**,⁷² see Figure 5) is a clinically approved orally bioavailable inhibitor of RTKs including MET, ALK, and AXL (IC₅₀ = 8, 20, and 294 nM, respectively, in biochemical assays). Pfizer developed this ATP-competitive inhibitor through a structure-based drug design strategy using the MET kinase domain for cocrystallization experiments.⁷² **12** potently inhibits cancer cell growth of cell lines harboring fusion variants and activating mutations of ALK⁷³ and also exhibits antiangiogenic properties.⁷⁴ Clinical studies of **12** in ALK-rearranged NSCLC rapidly demonstrated significant activity and clinical benefit,^{75,76} which led to its early approval by the FDA in 2011.⁷⁷ In a recent phase III clinical study of patients with ALK-rearranged NSCLC with metastasis in the brain, treatment with **12** was associated with systemic and intracranial disease control at 12 weeks of therapy.⁷⁸ However, following this period, acquired resistance to **12** was commonly observed, leading to the development of preexisting or new intracranial metastatic lesions.⁷⁸ Clinical studies of **12** against several cancers with ALK rearrangements or mutations including metastatic NSCLC, anaplastic large cell lymphoma, myofibroblastic tumor, and neuroblastoma are currently ongoing under different sponsorships.^{77,55}

Amuvatinib (**13**)⁷⁹ is a promiscuous type I kinase inhibitor targeting KIT, PDGFR1, FLT3, RET, and AXL developed by

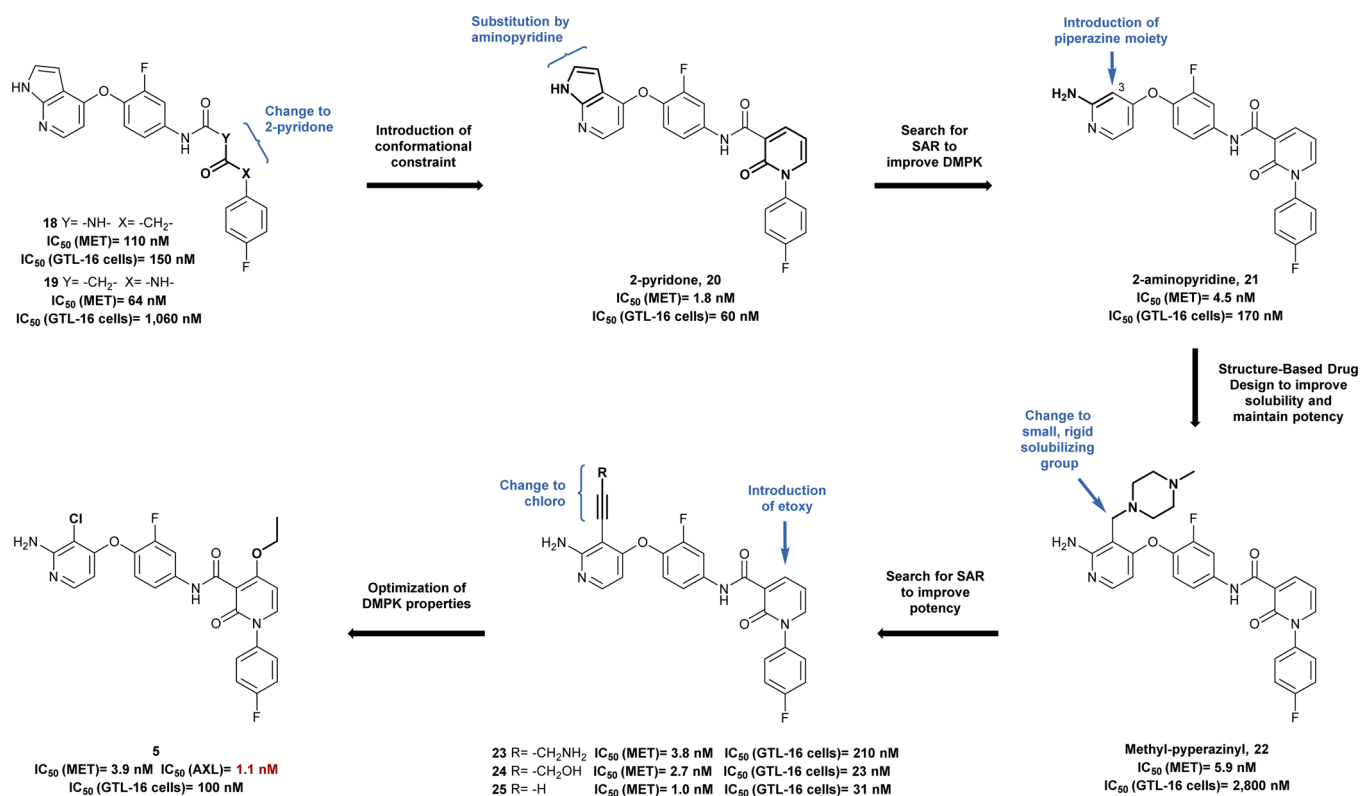


Figure 6. Development of the ATP-competitive (type II), orally available, dual MET/AXL inhibitor **5**.

Astex Pharmaceuticals. In MDA-MB-231 cells, it has been shown that this compound inhibits phosphorylation of AXL at 1 μ M.⁷⁹ Inhibition of AXL using **13** reversed EMT in murine breast cancer stem cells, reducing self-renewal and restoring sensitivity to chemotherapy.⁸⁰ After phase I evaluation in patients with solid tumors,⁸¹ **13** entered a phase II clinical trial in patients with NSCLC in combination with standard of care chemotherapy (platinum and etoposide, structures not disclosed). Despite favorable safety and preliminary clinical activity in the first stage of this phase II, Astex Pharmaceuticals announced discontinuation of its clinical development in 2012.

UNC2025 (**14**)⁸² is a promising preclinical candidate developed by Wang and co-workers at the University of North Carolina. It is a dual MER/FLT3 type I inhibitor (IC₅₀ < 1 nM, in cell-free assays for both kinases) that blocks AXL kinase activity with an in vitro IC₅₀ of 1.6 nM.⁸² In animal models, oral administration of **14** resulted in target inhibition in bone marrow leukemia cells.⁸² This compound derives from a previous MER/FLT3 inhibitor, UNC1062 (**35**),⁸³ see Figure 7), which was shown to reduce activation of MER-mediated downstream signaling in melanoma cells, inducing apoptosis in culture and inhibiting invasion.⁸⁴ On the basis of the remarkable capacity of **14** and some of its early derivatives to target AXL, its development program will be the focus of further discussions in following sections.

S49076 (**15**)⁸⁵ is an ATP-competitive inhibitor of MET, AXL, MER, and FGFRs (Figure 5) that targets AXL at low nanomolar levels both in vitro (IC₅₀ = 7 nM) and in cells (IC₅₀ = 56 nM, in murine embryonic fibroblasts engineered to express human AXL). The molecule was designed using a structure-based approach in the search for potent and selective MET inhibitors and its mode of binding inferred by X-ray resolution of cocrystal complexes of **15** analogs with MET.⁸⁵ Its

chemical structure closely resembles that of sunitinib (**16**),⁸⁶ a potent multitargeted RTK inhibitor approved for the treatment of different cancer indications that also inhibits AXL at the low nanomolar range (IC₅₀ = 9 nM).⁸⁷ **15** displays potent antiproliferative properties in MET- and FGFR2-dependent gastric cancer cells and inhibits migration of lung carcinoma cells. **15** potently inhibits phosphorylation of MET, AXL, and FGFRs in tumor xenograft models. Combination of inhibitor **15** with bevacizumab (structure not disclosed) in xenograft models of colorectal cancer resulted in near total suppression of tumor growth, and remarkably, **15** alone induced arrest of tumor growth in bevacizumab-resistant tumors. A phase I dose-escalation study of **15** (oral administration) is currently undergoing in patients with advanced solid tumors to establish its safety profile.⁸⁵

17 was the first kinase inhibitor to be intentionally designed to target the AXL RTK (see Figure 5).⁵³ It inhibits AXL in an ATP-competitive manner in the low nanomolar range (IC₅₀ = 14 nM) and displays significant selectivity (>40-fold) toward MER and TYRO3 (TAM family members) and other kinases including ABL and MET,⁵³ thus formally being the only selective AXL inhibitor reported to date. Hollande et al. showed that **17** is able to block AXL-dependent events in cells, including AKT phosphorylation, invasiveness of breast cancer cells, and the production of proinflammatory cytokines. Oral treatment with **17** in mouse xenograft models demonstrated a dose-dependent reduction of the EMT transcriptional regulator SNAIL and extended survival (>35%) in a MDA-MB-231 intracardiac mouse model of breast cancer metastasis.⁵³ Additionally, combination with cisplatin (structure not disclosed) enhanced reduction of liver micrometastases in a murine model. Originally developed by Rigel Inc.,⁵³ it was licensed to BerGen BIO in 2011 which is currently conducting

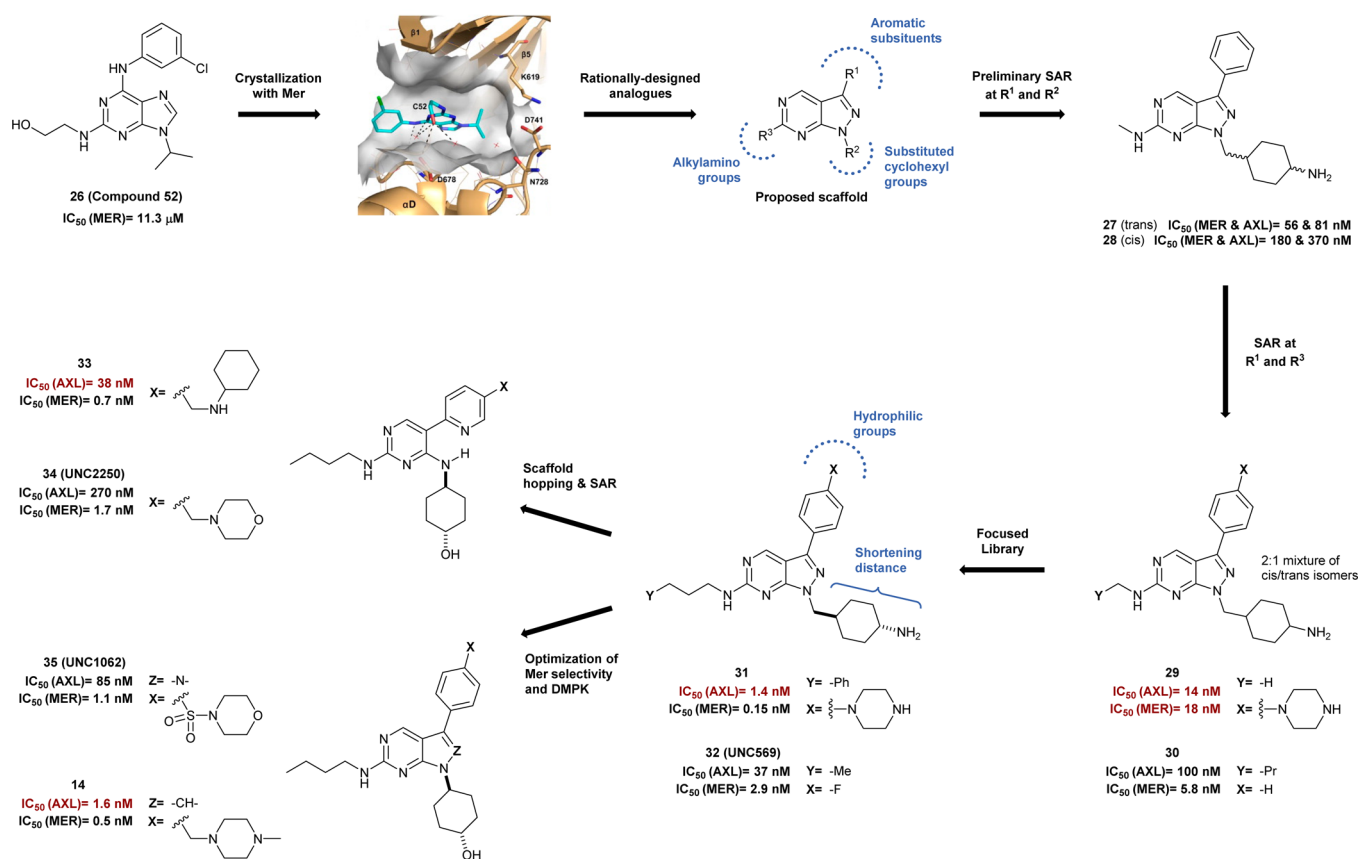


Figure 7. Medicinal chemistry campaigns for the discovery of TAM inhibitor **14** and related compounds by Wang and co-workers.^{82,83,95–97} MER-compound **52** cocrystal structure was adapted from Huang et al.⁹⁴ from *Journal of Structural Biology*, Elsevier, under the Creative Commons License Deed.

two phase I clinical trials,¹⁵ one of which received FDA orphan-drug designation for the treatment of AML. These trials are aimed at evaluating the safety and tolerability of the drug in AML and NSCLC patients when administered as a single agent and in combination with cytarabine and erlotinib (structures not disclosed), respectively.⁵⁶

3.3. Discovery of Dual MET/AXL Inhibitor 5. As discussed above, compound **5** is a MET inhibitor showing 3-fold greater potency for AXL than for MET in biochemical assays. The development of this drug candidate, which shares significant similarities with other type II kinase inhibitors such as **1** or **3** (see Figure 2), was carried out at Bristol Myers-Squibb. Encouraged by the potent inhibitory properties of pyrrolo[2,1-*f*][1,2,4]triazine-based compounds,⁸⁸ Schroeder, Borzilleri, and co-workers utilized this scaffold in the search for MET inhibitors. In silico modeling followed by library synthesis and in vitro screening led to potent MET inhibitors with poor pharmacokinetic properties.⁸⁹ Subsequent investigations focused on the substitution of the pyrrolotriazine scaffold by a pyrrolopyrimidine, which led to potent MET inhibitors **18** and **19** (see Figure 6).⁹⁰ While the malonamide derivative **19** inhibited the target with superior potency, the *N*-acylurea, **18**, showed higher antiproliferative activity in a MET-driven human gastric carcinoma cell line (GTL-16 cells).⁹⁰ The substitution of the malonamide group by a conformationally constrained 3-carboxamido-2-pyridone ring led to a dramatic increment in MET inhibition and superior antiproliferative activity in MET-dependent GTL-16 cells.⁹¹ 2-Pyridone derivative **20**⁹¹ also inhibited FLT3 and VEGFR2 at low

nanomolar concentration and demonstrated good in vivo efficacy in a GLT-16 xenograft model.

The success with the 2-pyridone fragment motivated the team to explore it on derivatives containing a 2-aminopyridine scaffold instead of the pyrrolopyridine one. *N*-Acylureas containing the 2-aminopyridine scaffold had previously demonstrated good inhibitory properties against MET but also potent inhibition of cytochrome P450 (CYP) isozymes.⁹⁰ Novel derivative **21**,⁵² which displayed high inhibition of MET kinase activity, served as a starting point for SAR studies. On the basis of the X-ray structure of derivative **20**,⁹¹ it was hypothesized that substitutions at the C3 position of the pyridine group could provide access to the ribose pocket of the ATP binding site. Different groups were therefore introduced in that position aiming to improve potency, solubility, and CYP inhibition profile. Introduction of an *N*-methylpiperazinyl-methyl moiety at C3 provided a soluble derivative (derivative **22**,⁵² Figure 6) with good on-target potency but low activity in cells. Substitutions by smaller, more rigid groups such as aminopropargyl, hydroxypropargyl, or unsubstituted propargyl groups led to a slight increase of MET inhibitory properties and superior antiproliferative activity in MET-dependent GLT-16 cells. Further optimization of DMPK properties resulted in inhibitor **5**, an orally available type II inhibitor with excellent half-life that inhibits AXL with an IC₅₀ = 1.1 nM (biochemical assays).⁵²

3.4. Medicinal Chemistry Campaigns for the Discovery of 14 and Related Compounds. Overexpression of the MER RTK is strongly associated with acute lymphoblastic

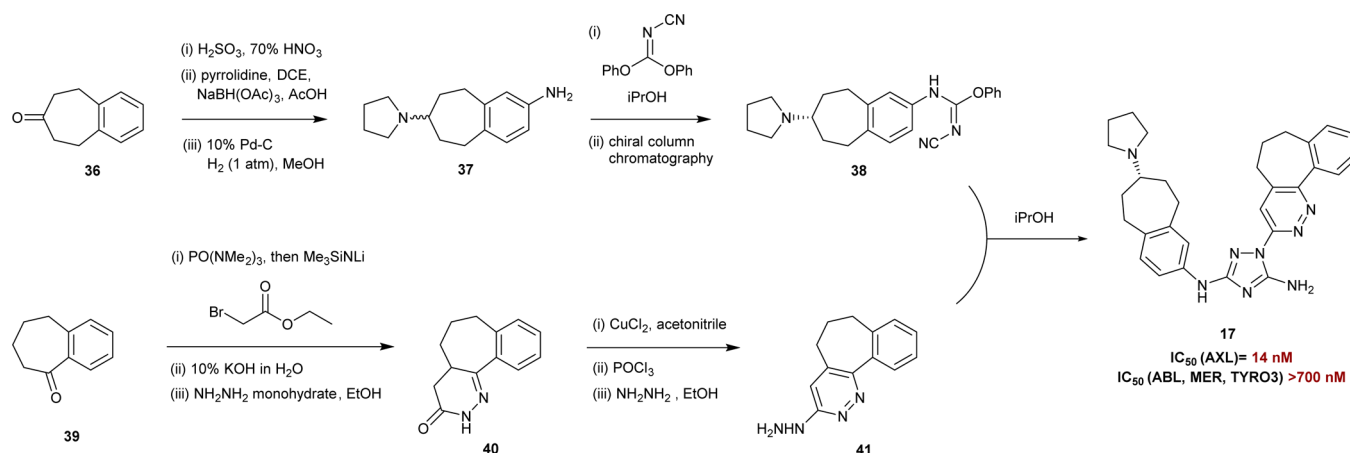


Figure 8. Synthesis of the specific AXL inhibitor 17 from commercial starting materials 36 and 39.⁹⁸

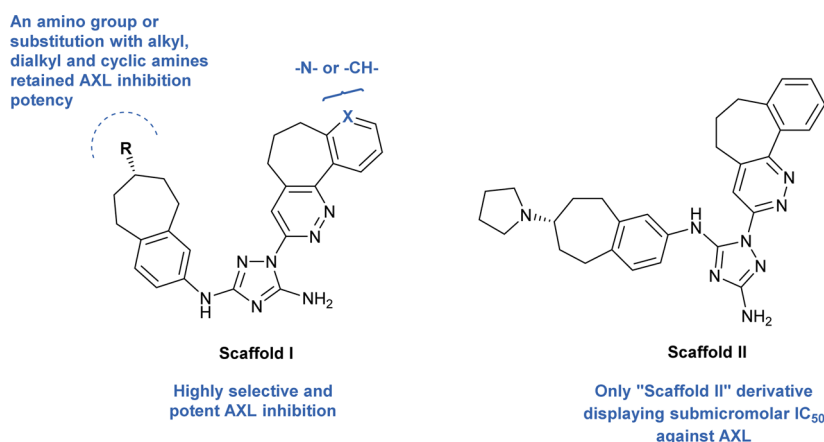


Figure 9. Chemical scaffolds investigated by Holland et al. in the search for specific AXL inhibitors.⁹⁸

leukemia (ALL). While MER is not expressed in normal human T lymphocytes at any stage of development, it is found ectopically expressed in over 50% of pediatric T-cell ALL.⁹² Compound 52 (26,⁹³ Figure 7), a polysubstituted purine derivative originally developed in Schultz' lab, is a weak inhibitor of MER ($IC_{50} = 11.3 \mu M$), the complex of which was cocrystallized with the MER protein and the structure resolved by Dhe-Paganon and co-workers.⁹⁴ This information was used by Wang and co-workers to create a docking model for MER inhibition.⁹⁵ On the basis of this model, they proposed a trisubstituted pyrazolopyrimidine scaffold to search for novel MER inhibitors (Figure 7). Preliminary SAR studies found that compounds 27 and 28,⁹⁵ both containing a 4-aminocyclohexylmethyl group in R_2 , displayed nanomolar activity against MER, with the trans isomer having 3-fold superior potency than the cis one. Given the similarities of MER with other members of the TAM subfamily, compounds were also screened against AXL and TYRO3. As shown in Figure 7, compounds 27 and 28 displayed slightly lower activity for AXL than for MER. Exploration of different alkylamino groups in the position R_3 of the ring showed that the larger the alkyl group, the higher the selectivity of the compound for MER. Introduction of the small methylamino group at R_3 generated highly potent AXL inhibitors such as compound 29,⁹⁵ the only compound of the series with higher affinity for AXL than MER. Among the range of aromatic substituents explored at the R_1 position, a 4-piperazinylphenyl group was found to be optimal for AXL

inhibition (see compounds 29 and 31 in Figure 7).⁹⁵ The trans isomers were found to be the most active ones across the libraries, with the most potent MER inhibitor (compound 31, $IC_{50} = 0.15 \text{ nM}$, in cell-free assays) possessing a phenylpropylamino group in R_3 . All compounds were found to be ATP competitive inhibitors. Due to its lower molecular weight, UNC569 (32,⁹⁵ with $IC_{50} = 2.9$ and 37 nM for MER and AXL, respectively) was the inhibitor chosen for further PD/PK analysis, showing inhibition of MER phosphorylation in cells ($IC_{50} = 141 \text{ nM}$ in human pre-B leukemia 697 cells) and good oral bioavailability (57%).

From this point two distinct medicinal chemistry campaigns were followed. A new family of potent MER inhibitors was developed by using a pseudo-ring replacement strategy (Figure 7).⁹⁶ From the new derivatives generated, compound 33⁹⁶ was the most potent against AXL ($IC_{50} = 38 \text{ nM}$ in biochemical assays), while the MER-targeting lead compound of the series, UNC2250 (34),⁹⁶ displayed good selectivity and PK profile, potent inhibition of MER phosphorylation in cells, and antiproliferative activity in a NSCLC cell line. Following studies on that scaffold led to UNC2881⁹⁷ (structure not disclosed), a potent highly selective MET inhibitor that demonstrated inhibition of platelet aggregation in collagen-stimulated human platelet-rich plasma. In parallel, optimization studies on the scaffold of inhibitor 32 proceeded toward increasing MER selectivity and DMPK properties (Figure 7). Studies resulted in the MER inhibitor 35,⁸³ which shows high

selectivity (>75-fold) over other TAM kinases, and the drug candidate **14**,⁸² a dual MER/FLT3 inhibitor with subnanomolar potency (but low selectivity over AXL and TYRO3) that displays excellent oral bioavailability (100%) and the capacity to inhibit MER phosphorylation in vivo in leukemic blasts of mouse bone marrow.

3.5. Discovery of AXL-Selective Inhibitor 17. As mentioned before, **17** is the most selective AXL inhibitor reported to date and the first one to progress to clinical trials based on its AXL inhibitory properties.¹⁵ The number of studies reported in the literature with this drug candidate is limited to biological studies.^{12,53,98,99} Apart from the original patent published by Rigel Inc. in 2010,⁹⁸ no other chemical report has yet become public. The patent application is thus the only source available to shed some light on how **17** was developed and review the structural requirements for the AXL inhibition activity of this interesting multicyclic [1,2,4]triazole-based compound.

As shown in Figure 8, the strategy to prepare inhibitor **17** consists of the generation of the polycyclic synthons **38** and **41** (by five- and six-step synthetic processes, respectively) from commercial starting materials, followed by a cyclocondensation reaction to form the central triazole of **17**.⁹⁸ This relatively straightforward strategy enabled Holland et al. to replace the pyrrolidine group on the cycloheptan ring of intermediate **37**⁹⁸ with different moieties. SAR analyses showed that an amino group was required in that position for inhibiting AXL (Figure 9), with dialkylated and cyclic amines providing the highest AXL inhibition activities.⁹⁸ When the amino group was masked as a carbamate, affinity for AXL was significantly reduced. It is also notable that halogens or amino groups functionalized with large cyclic structures were not well tolerated at that position. Twenty-six out of 56 compounds based on scaffold I exhibited submicromolar IC₅₀ values, showing the versatile potency of the scaffold. Three derivatives based on scaffold II were also synthesized, while only the one shown in Figure 9 exhibited AXL inhibition at the submicromolar range.⁹⁸

After the publication of the highly focused patent application that describes **17** and derivatives,⁹⁸ the same team at Rigel Pharmaceuticals Inc. filed divisional patent applications for new AXL inhibitors.^{100,101} The protected structures also contain the central triazole group representative of this unique class of compounds, while the chemical variety of substituents used in each of those patents have been significantly expanded. It is therefore likely to expect that the discovery of novel selective AXL inhibitors will be reported at some point in the future.

3.6. General Considerations with Regard to the Use of Current AXL Inhibitors in Cancer Treatment. The interest of Pharma and the research community in AXL inhibitors as anticancer agents has dramatically increased over the past 10 years. Given the amount of evidence supporting the role of AXL in cancer and the increasing number of AXL-targeting candidates entering clinical trials, it is likely that such interest will continue to rise in the years to come. After a review of the state-of-the-art, however, it seems clear that the main barrier that could potentially block the generation of clinically useful AXL inhibitors is their selectivity profile. The majority of inhibitors of AXL kinase activity also inhibit other protein kinases, preferentially RTKs. This is due to the considerable similarities among the intracellular kinase domains of RTKs,¹⁰² which are very significant not only within the members of the TAM subfamily (TYRO3, AXL, and MER), but also among other RTKs such as MET, FLT3, RON, and Aurora A/

B.^{67,71,103,104} Consequently, the discovery of ATP-competitive kinase inhibitors displaying cross-reactivity for multiple RTKs has been and will continue to be a widespread phenomenon, whereas selectivity over other groups of kinases should be expected.¹⁰⁵ Due to the complexity of cancer pathology, a certain level of promiscuity may actually become an advantage in cases where inhibiting more than one target can lead to a synergistic effect for treatment. For instance, this may be one of the reasons behind the remarkable clinical response mediated by the VEGFR2/MET/AXL inhibitor **2** in patients with advanced renal cell carcinoma.⁵⁸ However, in other cases a poor selectivity profile will result in the inhibition of kinases that are essential for physiological functions and, consequently, in severe side effects.¹⁰⁶ This issue may become a challenging one, since an AXL inhibitor will rarely be employed in the clinic on its own, and drug combinations usually lead to incremental adverse effects.

Interestingly, across the list of inhibitors described in this article (Table 1), something can be concluded: contrary to the general assumption, type II kinase inhibitors provided no benefit in terms of selectivity over type I inhibitors. A potential way to improve the selectivity profile of AXL inhibitors would be to focus on the development of either allosteric or irreversible inhibitors.^{107,108} Nonetheless, the unique properties of inhibitor **17** proves that ATP-competitive inhibitors with adequate selectivity profile and AXL potency can certainly be generated.

Due consideration to the immunoregulatory role of AXL, MER, and TYRO3 is also essential to understand the limitations of a potential anti-AXL therapy. Studies by Lu and Lemke reported that TAM triple knockout mice develop broad-spectrum autoimmunity and a severe lymphoproliferative disorder.¹⁰⁹ Bosurgi et al. showed that ablation of AXL and MER signaling in mice favors a tumor-promoting environment in the colon by increasing production of proinflammatory cytokines and reducing clearance of apoptotic neutrophils in the intestines.¹¹⁰ On the other hand, the role of TAM receptors in diminishing the innate immune response makes their inhibition an attractive mechanism to reverse the immunosuppressive tumor microenvironment.⁶² Furthermore, it has been demonstrated that tumor-macrophage cross-talk is responsible for stimulating the macrophage secretion of GAS6 in the tumor microenvironment, which in turn fosters tumor growth by activation of TAM RTKs, particularly AXL.¹¹¹ Consequently, while chronic inhibition of TAM family members, particularly MER, could eventually result in autoimmune side effects,^{19,24} the benefit provided by the temporary use of AXL inhibitors for cancer treatment would considerably overcome the associated risks. To minimize detrimental autoimmune responses, it may be particularly useful to generate further AXL inhibitors with selectivity over the other TAM members MER and TYRO3, the role of which seems to be more important in immunoregulation.¹⁹

4. CONCLUSIONS

The shift of the Pharma industry and the medical community toward personalized cancer medicine and genotype-guided therapeutic intervention is causing an ever-increasing need for both single- and multitargeted inhibitors.¹¹² While the number of treatable patients can significantly decrease with the use of drugs that selectively target proteins involved in specific cancer types, the effectiveness and safety of these targeted therapies can be far greater. However, since cancer survival and

progression are typically driven in most malignancies by more than one protein or signaling pathway, the clinical use of targeted monotherapies normally becomes insufficient to fully control or eliminate the disease.¹¹³ Furthermore, resistance mechanisms typically arise to both chemotherapeutics and targeted therapies through mutations or activation of additional proteins or signaling pathways.¹¹⁴ Due to their pathological and etiological complexity, pharmacological interventions based on combination strategies will be essential to effectively treat those cancers.^{113,114} However, the shortage of highly selective targeted agents against many relevant oncotargets is limiting the development of drug combinations with acceptable safety profiles. As more and more proteins involved in neoplasia and/or chemoresistance mechanisms are uncovered and their roles elucidated, the need for targeted inhibitors becomes ever greater.

The prominent role of AXL in the survival, spread, and mechanisms of drug resistance in different solid and blood cancers strongly supports the choice of AXL inhibition as an anticancer strategy, especially for advanced disease.^{16–19,115} Consequently, various drug candidates targeting AXL are currently under clinical development, and more are expected to come. Preliminary clinical evidence in a NSCLC patient with AXL gene amplification receiving treatment with AXL/MET inhibitor **4** has shown that AXL abnormalities may be clinically relevant oncogenic drivers in certain forms of NSCLC.⁶³ These particular cancer types could represent the primary clinical indication for AXL-targeted monotherapy. However, since cases where AXL is the main driver of the cancer are rare, AXL inhibitors would be most beneficial as combination partners. For example, strong evidence indicates that AXL inhibition could significantly benefit anti-EGFR, anti-HER2, or anti-VEGFR therapies.^{26,35,44} AXL inhibitors also have the potential to impact cancer metastasis, but translating this into clinical trials is currently problematic. Therefore, at the moment AXL inhibitors show more promise in the treatment of resistant disease. AXL upregulation is one prominent mechanism whereby chemoresistance develops in different cancers,^{13,14} therefore strongly indicating that AXL inhibitors could synergize in the clinic with standard-of-care chemotherapeutics such as cisplatin (structure not disclosed). If AXL targeting drugs with adequate selectivity profile are developed and synergistic drug combinations established, they could provide cancer patients with new therapeutic options to fight the latest and most aggressive forms of cancer.

As reviewed in this article, there are currently just a few drug candidates that have AXL as its primary target and only one reported clinical candidate can be considered as a selective AXL inhibitor. One of the challenges we face for the de novo design of AXL inhibitors is the lack of crystallographic data for the kinase domain of the AXL RTK. Given the growing demand for small molecule inhibitors targeting AXL activity, this limitation will certainly be addressed in the near future. Nonetheless, until this information becomes available, ligand-based approaches provide one useful route to develop AXL inhibitors with improved properties. This comprehensive review of the state-of-the-art is an effort to promote and facilitate medicinal chemistry activities in such a direction.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

RTK, receptor tyrosine kinase; TAM, TYRO3, AXL, and MER; GAS6, growth arrest-specific 6; EMT, epithelial–mesenchymal transition; CML, chronic myeloid leukemia; PtdSer, phosphatidylserine; CpG, cytosine–phosphate–guanine; RNAi, RNA interference; ER+, estrogen receptor-positive; DFG, aspartate–phenylalanine–glycine; ATP, adenosine triphosphate; NSCLC, non-small-cell lung carcinoma; IC₅₀, half maximal inhibitory concentration; FDA, Food and Drug Administration; NK, natural killer; AML, acute myeloid leukemia; CYP, cytochrome P450; ALL, acute lymphoblastic leukemia; SAR, structure–activity relationship; DMPK, drug metabolism and pharmacokinetic; AACR, American Association for Cancer Research

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